SEQUENTIAL ULTRASTRUCTURAL CHANGES DURING THE IN VITRO CULTIVATION OF *TRYPANOSOMA CRUZI*

C. Duran and F. Tejero.
Laboratorio de Biología Funcional de los Kinetoplastida. Sección de Parasitología. Instituto de Zoología Tropical. Facultad de Ciencias. Universidad Central de Venezuela. Caracas. Venezuela.
Dr. Felix Tejero. Apartado 60006 Chacao. Caracas 1060. Venezuela.
Tlf.: +58-0412-975-8664. Fax: +58-0212-264-5213. E-mail: felixtejero@cantv.net.

Abstract

The development of *Trypanosoma cruzi* is determined by particular attributes as changes in shape probably connected to variations in density population. In this work we approach this topic by the study of the ultrastructural transformations of *T. cruzi* in axenic cultures. Results indicate *T. cruzi* growth reaching the maximum population peak on day 19th and a continuous population decrease from day 21. Also, point out a continuous reduction in the glucose concentration of the culture medium; however, glucose does not disappear completely. In addition, the results show that the ultrastructural characteristics of the *T. cruzi* epimastigotes vary as the time of culture go by. The changes include mitochondrial and nuclear modifications, as well as dispersion of mitochondrial profiles, nuclear compacted heterochromatin and increasing number of autophagic vacuoles. Subcellular changes described in this work could represent a *T. cruzi* adaptable answer induced by the culture medium conditions.

Resumen

El desarrollo de *Trypanosoma cruzi* es determinado por atributos particulares como cambios de forma, probablemente unidos a variaciones en la densidad poblacional. En este trabajo nos aproximamos al tópico mediante el estudio de las transformaciones ultraestructurales de *T. cruzi* en cultivos axénicos. Los resultados indican que el crecimiento de *T. cruzi* alcanza el pico máximo de densidad poblacional el día 19 y que a partir del día 21, la población disminuye continuamente. También señalan una reducción continua de la concentración de glucosa en el medio de cultivo; sin embargo, la glucosa no desaparece completamente. Los resultados, además muestran que las características ultraestructurales de los epimastigotes de *T. cruzi* varían en la medida que transcurre el tiempo de cultivo. Los cambios incluyen modificaciones nucleares y mitocondriales, así como dispersión de los perfiles miotocondriales, compactación de la heterocromatina nuclear y números crecientes de vacuolas autofágicas. Los cambios subcelulares descritos en este trabajo, podrían representar una respuesta adaptativa del *T. cruzi* inducida por las condiciones del medio de cultivo.

Keywords: *Trypanosoma cruzi*, sequential ultrastructure, culture.

Introduction

The life cycle of *Trypanosoma cruzi,* the etiologic agent of Chagas' disease, requires the contribution of two hosts, an invertebrate (Reduviidae: Triatominae), and a mammal [1]. The development of *T. cruzi* in a particular host is set by an emphasized change in shape [2] associated with density-dependent events [3]. However, the relationships among population densities, glucose consumption, and ultrastructural changes are not properly understood. We consider that the connection between population density oscillations and changes in the

concentration of energy supply molecules can be upheld, in addition to the mathematical model cited in reference [3], by ultrastructural analysis.

In this work we study the sequential ultrastructural changes appearing in *T. cruzi* during axenic *in vitro* culture, and speculate about its association with the population density and the glucose consumption.

Material and Methods

5×10^6 axenic *T. cruzi* "Y" strain epimastigotes harvested in mid log phase were seeded in disinfected Erlenmeyer flasks containing sterile BHI biphasic medium [4].

The population density was estimated every other day, by means of a Neubauer chamber. Same days, 50 □l samples of the overlay were centrifuged (347×g/25 min), and the supernatant was used to find the concentration of glucose espectrophotometrically at 500- 510 nm [5]; pH was also specified.

The sediments were fixed with Karnovsky's solution [6] and post-fixed with osmium tetroxide for routine transmission electron microscopy [7]. Carved epoxy resin blocks were sectioned (Porter-Blum MT2), and ultrathin sections were contrasted [8,9]. Permanent record was done with a Jeol Jem-1200 electron microscope (100kV).

Results and Discussion

T. cruzi growth exhibits the typical patterns of a normal distribution curve, reaching the maximum population peak on day 19th with a parasitic charge around 8´109 flagallates/ml, remaining alive until day 33. The population decrease continuously from day 21, Figure 1.

The glucose concentration in the overlay of the culture medium shows a continuous decrease in comparison with the control cultures, Figure 2. Glucose in the BHI overlay is not completely consumed, probably because since day 21 the population of the parasites, decrease uniformly until day 33.

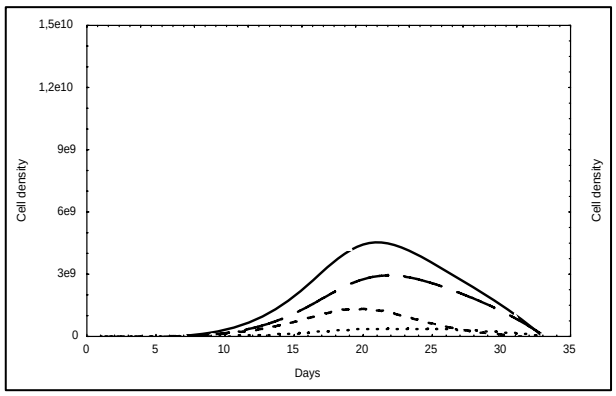

Figure 1. Population density and morphotypes in *Trypanosoma cruzi*. BHI culture. Symbols: ——: density; — —: epimastigotes; – – –: sphaeromastigotes; ····: micromastigotes. Data adjusted by jeans of polynomial regression.

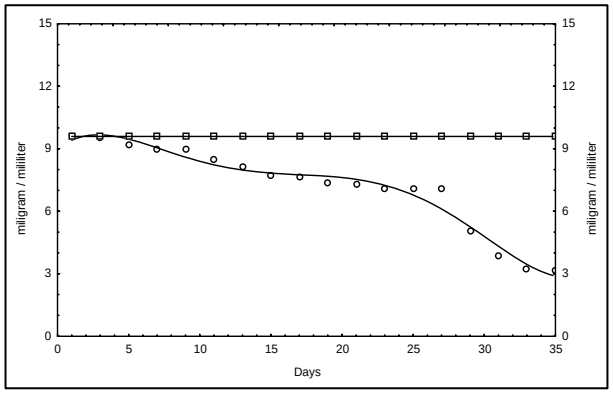

Figure 2. Changes in the concentration of glucose in cultures of *Trypanosoma cruzi* maintained in BHI medium. Symbols, ○: [glucose]; □: control.

The ultrastructural characteristics of the *T. cruzi* epimastigotes, maintained in BHI medium, vary as the time of culture go by.

The changes include mitochondrial and nuclear modifications, Figure 3. In addition, dispersion of mitochondrial profiles, nuclear compacted heterochromatin and increasing number of autophagic vacuoles are beheld, Figure 4.

The culture medium damage associated to the growth of prokaryote [10] and eukaryote [11] is a well known fact. Such circumstance, joined to quantitative ultrastructural analysis [12], forces the research to a sequential ultrastructural approach relating subcellular changes to population density and chemical variations in the culture medium.

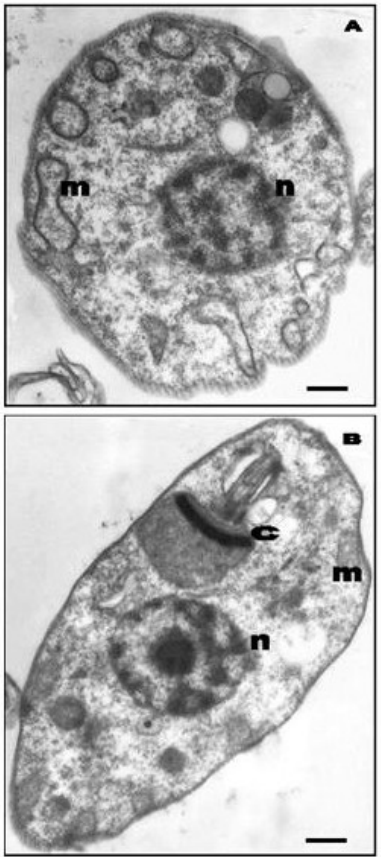

Figure 3. Ultrastructure *of Trypanosoma cruzi*. A. Modified nucleus (n) and mithocondrial profiles (m). B. Kinetoplast (c) nucleus (n) and mithocondrial profiles (m). 15000X

Our results show a poor mitochondrial cristae development, making clear a mitochondrial slowness as the time goes by. Some authors have proved a direct time dependant link between the decrease in the number of mitochondrial profiles and the reduction of the mitochondrial activity in *T.* rangeli cultures [13]. Changes in the *T. cruzi* mitochondrial area, suggesting its

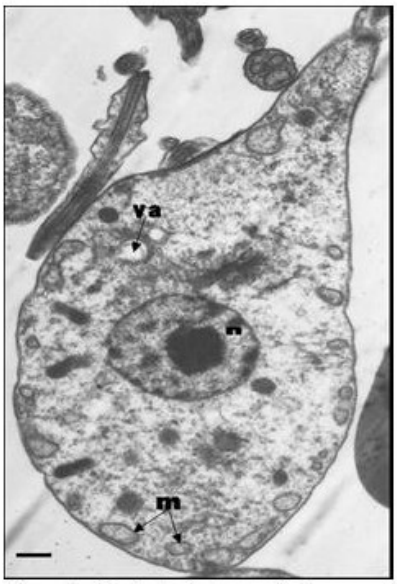

Figure 4. Ultrastructure *of Trypanosoma cruzi*. Compact heterocromatin in the nucleus (n). Dispersion of mitochondrial profiles (m). Autopahgic vacuoles (va) 8000X. Bar=1µm.

connection with chemical modifications in the culture medium have already calculated [12].

In this work, the scanty mitochondrial cristae development is associable with high concentrations of glucose in the culture medium, which could decelerate mitochondrial synthesis and, on the other hand, \

accelerate glicosomal activity. Such supposed mitochondrial deceleration would be connected to the progressive diminution of mitochondrial profiles per squared micrometer. Many heteroxenous parasitic protozoa have adaptations favoring population density increments in the host, adapting its oxidative phosphorylation to the metabolism of the vertebrate host [14]. However, other author indicates that there are no significative changes in the *T. cruzi* mitochondrial cristae along its developmental cycle [15]. Such finding is supported by reports indicating no differences in the respiratory metabolism of epimastigotes, trypomastigotes and amastigotes of *T. cruzi* [16,17].

We suggest a relationship between nuclear and kinetoplast changes and metabolic activity. Such

connection could be linked to energetic saving and adaptations to the environmental stress. Considering the diminution in heterochromatin area, we presume a decrease in the exportation protein synthesis and cell division.

Conclusions

The cultures of *T. cruzi* exhibit a normal *in vitro* growth with a continuous reduction in the medium glucose concentration without completely consumption.

The ultrastructural characteristics of the *T. cruzi* epimastigotes vary as the time of culture go by. The changes include mitochondrial and nuclear modifications, as well as dispersion of mitochondrial profiles, nuclear compacted heterochromatin and increasing number of autophagic vacuoles.

Subcellular changes described in this work could represent an adaptable answer of the parasite induced by environmental (culture medium) conditions.

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